

Methods The SNQ tri-peptide from i2 was engineered into *H pylori* strain 60190 (VacA s1/i1/m1) to make strain 60190/SNQ. The vacuolating activities of 60190/SNQ and wild type strains were compared by incubating AGS cells with broth culture supernatants, and counting vacuolated cells in random fields by microscopy. To allow further structure-function characterisation of i-region variants, we modified a recombinant VacA system² by expressing VacA p33 and p55 subunits in *Escherichia coli* and purifying both under denaturing conditions by metal affinity chromatography, then refolding by stepwise dialysis. Recombinant VacA was tested for vacuolating activity and previously described effects on Jurkat T-cells.³

Results Introduction of the i2-specific SNQ insertion into i1 VacA in the *H pylori* 60190 strain resulted in a 1.8-fold decrease in native toxin vacuolating activity on AGS cells lines (pH pylori—they induced extensive vacuolation of gastric epithelial cell lines at concentrations down to 0.2 µg/ml. The recombinant toxin also inhibited proliferation of, and IL-2 production by Jurkat cells. Engineering the SNQ insertion into recombinant i1 VacA resulted in modest reductions in the stability of the toxin.

Conclusion A naturally-occurring three amino acid insertion in the VacA i-region renders the toxin less active in vitro. We speculate that this small insertion is a major contributor to the reduced association with cancer of strains with type i2 VacA. Recombinant VacA toxin is highly active on both epithelial and T cells and can be genetically modified to explore VacA structure-function relationships. We aim to use this system to further characterise the structure and function of i-region variants.

Competing interests None declared.

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PWE-172 FACTORS INFLUENCING MALNUTRITION AFTER CURATIVE GASTRECTOMY FOR GASTRIC CARCINOMA

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Introduction Patients with gastric carcinoma often suffer from malnutrition after curative gastrectomy. It is difficult to identify patients who require further nutritional support as part of their long-term post-operative care because factors influencing weight loss are unknown, and it is also inefficient to directly evaluate the nutritional status of every patient using available screening tools. This study aims to identify factors influencing weight loss after curative gastrectomy, which can be used to efficiently identify at-risk patients to improve outcomes.

Methods 163 patients (71.8% of those eligible) who underwent curative gastrectomy from 2000 to 2010 at the National University Hospital were identified from a prospectively maintained database. Factors predicting severe malnutrition at 6 months post-gastrectomy (defined as ≥10% loss of pre-operative weight), and factors influencing gain/plateau vs further weight loss over the following 6 months in the patients who were malnourished at 6 months post-gastrectomy were identified with multiple logistic regression.

Results Severe malnutrition was present in 52.8% (n=86) at 6 months post-gastrectomy. For these patients, those who had

anaemia (OR 3.662, p=0.006), gastrointestinal bleeding (OR 2.532; p=0.038) or weight loss (OR 1.052; p=0.010) at presentation, histopathologies of poorly and moderately differentiated tumours (OR 3.632 and 1.757 respectively; p=0.029), adjuvant therapy (OR 8.464; p=0.000), and those requiring step-down care upon discharge (OR 5.739; p=0.021) were at increased risk of weight loss. However, male gender (OR 0.288; p=0.012), vomiting at presentation (OR 0.219; p=0.014), and neo-adjuvant therapy (OR 0.225; p=0.027) were associated with decreased risk. Age, race and operation length were not significant factors at 6 months. Of the patients who were malnourished at 6 months, 54.9% (n=39) developed further weight loss over the subsequent 6 months. Pre-operative total lymphocyte count (OR 2.141; p=0.033) and tumour size (OR 1.235; p=0.054) were associated with increased risk. Age, gender and race were not significant factors at 12 months.

Conclusion Malnutrition is a significant problem post-gastrectomy. More nutritional care should be provided to patients presenting with acute presentation, poor tumour biology and those requiring adjuvant therapy and step-down care as these are risk factors at 6 months. At 1 year, patients who initially had larger tumour sizes and higher pre-operative total lymphocyte counts are at greater risk of continued weight loss, and should receive further nutritional support as part of their long-term post-operative care.

Competing interests None declared.

PWE-173 INCREASED CCL20 AND CCR6+ REGULATORY T-CELL RESPONSES IN THE *HELICOBACTER PYLORI* INFECTED HUMAN GASTRIC MUCOSA

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Introduction *Helicobacter pylori* (*Hp*) infection may result in peptic ulcers or gastric cancer. Disease risk is associated with more virulent strains, such as those with the *cag* pathogenicity island (*cagPAI*), which induce higher levels of gastritis. Disease is also associated with an insufficient anti-inflammatory regulatory T-cell (Treg) response to *Hp*. Preliminary studies of circulating T-cell adhesion molecule expression highlighted an increased frequency of CCR6+ Tregs in the blood of *Hp*+ patients. This chemokine receptor is expressed by both T-helper 17 (Th17) cells and Tregs. It has been demonstrated that the *Hp*-infected human gastric mucosa contains significantly raised concentrations of the CCR6 ligand, CCL20. We hypothesised that CCL20 may play a role in the migration of Tregs to the infected mucosa, and therefore aimed to investigate the mechanisms by which CCL20 expression is induced by *Hp*, and to quantify and characterise gastric mucosal CCR6+ Tregs.

Methods 24 *Hp*+ and 34 *Hp*- patients attending the Queen's Medical Centre, Nottingham, donated gastric biopsies and peripheral blood with informed consent and ethics approval. Isolated CD4 cells were stained for Treg markers (CD25^{hi}, FOXP3⁺, CD127^{lo}) and CCR6, prior to analysis by flow cytometry. Gastric epithelial cell lines (AGS, MKN28 and MKN45) were cultured with *Hp* (Wild type, and the following null mutants: Δ *cagA*, Δ *cagE*, Δ *vacA* and Δ *slit*) at a range of multiplicities of infection (MOI), with or without chemical inhibitors, for up to 48 h. CCL20 in culture supernatants was quantified by ELISA.

Results CCL20 levels were threefold higher in biopsies from *Hp*+ patients than *Hp*- patients (p=0.015). >80% of Tregs extracted from gastric biopsies were CCR6+, and 3.5-fold higher numbers of Tregs were present in samples from infected compared to uninfected patients (p=0.050). twofold higher proportions of Tregs in the peripheral blood of *Hp*+ patients were CCR6+ (p=0.021). In cell lines, *cagPAI*+ *Hp* strains induced a dose-dependent increase in

CCL20 production, with a sixfold increase after 24 h at MOI 1 ($p=0.01$). The $\Delta cagE$ ($p<0.01$) and Δsli ($p<0.01$) but not $\Delta cagA$ ($p=0.18$) inactivations reduced CCL20 induction by multiple *Hp* strains, indicating CagA-independent, *cagPAI*-dependent signalling.

Conclusion *Hp* induces CCL20 production by gastric epithelial cells in a *cagPAI*-dependent manner. Higher gastric CCL20 levels in *Hp*+ patients correspond to increased gastric infiltration of CCR6⁺ Tregs and to the proportions of these cells in the peripheral blood. We speculate that CCL20/CCR6 is the main homing system for Tregs to the stomach in *Hp* infection and thus central to pathogenesis. Migration assays are being performed in vitro prior to in vivo studies in mouse models.

Competing interests None declared.

PWE-174 PROTON PUMP INHIBITORS (PPI) USE AND CLOSTRIDIUM DIFFICILE INFECTION: GUILTY OR INNOCENT BYSTANDER?

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Introduction PPIs have been implicated in predisposing to *Clostridium difficile* infection by causing hypochlorhydria. A 5–10% asymptomatic toxin burden in the community is also reported. The previously voluntary reporting of *C difficile* infection in UK became mandatory for all age-groups in April 2007. Aim: To compare the pattern of PPI prescribing & *C difficile* rates in Rotherham (industrial town in Yorkshire; pop 250k) and England (50mill.) over a 7-year period to identify any association at a community level.

Methods Retrospective population study of seven consecutive years (2004/2005–2010/2011) on data of annual PPI prescriptions & *C difficile* incidence (both/100k pop.) in Rotherham primary care trust (PCT) and all England PCTs combined obtained from our microbiology department, Rotherham PCT, NHS Information Centre and UK Health Protection Agency. PPI prescribing trends and *C difficile* rates (irrespective of PPI use) in each year was compared between the two groups as % difference (Rotherham vs England: + =higher %, - =lower %).

Results (Abstract PWE-174 table 1) PPI prescription: Prescription rates have risen steadily in both cohorts over the study period. Rotherham had higher rate throughout study period but gap with England in % terms has steadily narrowed. *C difficile* rates: Rotherham rates were much higher till 2006/2007, reversed dramatically in 2007/2008, continuing to fall for next 2 years. England rates peaked in 2007/2008 and fell steadily from 2008/2009 with hardly any gap in 2010/2011.

Abstract PWE-174 Table 1 PPI prescribing and *C difficile* rates in Rotherham (R) vs all-England (E)

	Rates/year/100 k population						
	2004/ 2005	2005/ 2006	2006/ 2007	2007/ 2008	2008/ 2009	2009/ 2010	2010/ 2011
R PPI	53 566.0	60 217.0	66 974.0	75 948.0	80 554.0	86 641.0	92 829.0
E PPI	39 715.6	45 501.1	51 505.0	59 543.6	66 800.4	73 876.3	80 377.4
R/E difference (%)	+26	+24	+23	+22	+17	+15	+13
R <i>C difficile</i>	120.0	150.4	144.4	88.0	54.0	38.0	40.4
E <i>C difficile</i>	80.8	94.0	102.6	111.0	72.2	51.2	43.4
R/E difference (%)	+33	+38	+29	-26	-34	-35	-1

Conclusion Strict hand hygiene in hospital and microbiologist-controlled prudent antibiotic use in hospital and community from 2002/2007 seem to have resulted in a marked fall in *C difficile* rates in Rotherham from 2007/2008. We presume that similar measures, gradually introduced in the rest of England during 2007, account for the more widespread but less steep fall in the England infection rates from 2008/2009. The 7-year community level data suggests a mere association rather than true cause-effect relation between *C difficile* rates and PPI use in the past. Any potential risk from PPI use seems to be offset by rigorously applied hand hygiene (secondary care) and careful antibiotic prescribing (primary and secondary care) as evidenced by falling infection rates, despite rising PPI prescribing, since 2007/2008.

Competing interests None declared.

PWE-175 DUODENAL EOSINOPHILIA AND EARLY SATIETY IN FUNCTIONAL DYSPEPSIA (FD): CONFIRMATION OF A POSITIVE BIOMARKER ASSOCIATION FOR FD IN AN AUSTRALIAN COHORT

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Introduction Functional dyspepsia (FD), defined by unexplained pain or discomfort centred in the upper abdomen, affects 15% of the population. Diagnosis and treatment of FD based on the symptom-based Rome III criteria remains challenging. Recently, subtle eosinophilic inflammation in the duodenum has been implicated in the pathophysiology of FD in adults based on a Swedish case-control study.¹ Specifically, increased eosinophils in early satiety and post-prandial distress have been replicated in the UK,² in paediatric dyspepsia in the USA³ and in post-infectious FD in Japan,⁴ but the association remains controversial. The aim of this study was to characterise upper gastrointestinal tract pathology in an Australian cohort and correlate this with available clinical data in FD cases and controls.

Methods Patients prospectively referred for an upper gastrointestinal endoscopy (n=55; mean age, 49.6 years; 61.8% female) were entered to the study (with informed consent), stratified to FD cases (n=33) and controls (n=22) by Rome II criteria and completed a validated Bowel Symptom Questionnaire. Two blinded independent observers assessed the eosinophil count in five high power fields, in the duodenal bulb (D1) and second part (D2). *H pylori* status was assessed by gastric histology. Associations with clinical symptoms were assessed by Mann–Whitney U test.

Results Cases and controls were demographically similar. There was a significant increase in eosinophils in D1 vs D2 for both cases ($p=0.0003$) and controls ($p=0.008$). Abdominal pain was associated with eosinophilia in both D1 ($p=0.03$) and D2 ($p=0.007$). Duodenal eosinophilia was significantly increased in subjects experiencing early satiety, ($p=0.015$). This association remained after exclusion of coeliac disease (n=2) and *H pylori* (n=9, 16%) ($p=0.04$) in D2 but not in D1. Subjects who “felt food staying in their stomach” similarly had increased D2 eosinophilia ($p=0.002$); this remained significant on exclusion of coeliac disease and *H pylori* ($p=0.004$). Smoking was also associated with eosinophilia in D2 ($p=0.008$).

Conclusion Data supporting subtle duodenal eosinophilia in subsets of FD has become credible. The potential role of duodenal eosinophils as biomarkers has implications for diagnosis and therapeutic trials.

Competing interests None declared.